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WESTERN EQUINE ENCEPHALITIS ANTIBODY TYPE-VARIATIONS IN SERA FROM U. S. MILITARY RECRUITS

Robert W. Lyons

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WESTERN EQUINE ENCEPHALITIS ANTIBODY TYPE-VARIATIONS IN SERA FROM U. S. MILITARY RECRUITS.

by

Robert W. Lyons

A thesis submitted to the Faculty
of the Yale University School of Medicine
in partial fulfillment of the requirements
for the degree of Doctor of Medicine

Department of Epidemiology and Public Health
Yale University School of Medicine

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INTRODUCTION

Objectives of this study

Recent work has shown that western equine encephalitis virus (WEE) exists in at least three antigenic phases. The purpose of this study was to determine if these antigenic phase variations are manifest in the WEE serum antibodies of individuals presumably infected with various phases of the virus sometime during their lifetime. Attempts were also made to correlate the geographic distribution of the virus strain type (phase) with antibody type.

For these purposes hemagglutination-inhibition (HI) tests and neutralization (Neut) tests were performed with two antigenic variants of WEE virus using sera from U. S. Military recruits known to possess HI antibodies to WEE.

Antigenic Classification of Arboviruses

An arthropod-borne virus (arbovirus) is defined as a virus which can multiply in the body of an arthropod and which can be biologically transmitted to vertebrates by the anthropod. The arbovirus does not cause any known pathological change in the arthropod vector but may cause illness in the vertebrate host (1).

In 1901 Reed isolated the first such virus, yellow fever, which he showed to be carried by *Aedes aegypti* and

transmitted by this mosquito to man (2). Since that time more than 150 viruses have been classified as arboviruses (3). Many of these cause no known disease, and indeed many have not been isolated from arthropods (4-6). These viruses have been classified as arboviruses primarily because of their serological relationships. Antigenic relationships among the arboviruses have become more and more important as their number grows, and more detailed subdivisions become necessary.

Among the first investigators to note antigenic similarities among the arboviruses was Smithburn (7), who in 1941 found that West Nile (WN), St. Louis encephalitis (SLE) and Japanese B encephalitis (JBE) viruses would cross react serologically in the neutralization test. Two years later it was reported by Havens (8) that eastern equine encephalitis (EEE) virus would cross react with WEE in the complement-fixation test. Casals (9) in 1944 applied several tests to the viruses of Russian spring-summer encephalitis (RSSE), louping ill (LI), WEE, JBE, SLE, and WN. He found RSSE and LI to be closely related by the complement-fixation test but not by the mouse intercerebral cross resistance test. He found WEE to be unrelated to these viruses by any test.

Serological relationships between arboviruses were clarified by Casals and Brown (10) in 1954 when they reported classifying sixteen arboviruses into two groups, A and B,

according to their cross reactivity in the hemagglutination-inhibition (HI) test. Mouse immune serum for any serogroup A virus would inhibit the agglutination of chick red cells by all serogroup A viruses. Any serogroup B immune serum would do the same for all serogroup B viruses, but there was no cross inhibition between the immune sera of one serogroup and a virus of the other. They found that the best hemagglutination with serogroup A viruses was at 37°C at pH 6.4, while serogroup B viruses hemagglutinated best at either 4°C or 22°C at pH 7. They placed EEE, Semliki forest (SF), Sindbis, Venezuelan equine encephalitis (VEE), and WEE viruses into serogroup A and dengue types 1 and 2, Ilheus, JBE, LI, Ntaya, SLE, RSSE, Uganda S, WN, and yellow fever viruses into serogroup B.

In later work Casals (1,6) described the serogroup C viruses. These were five viruses isolated from animals at the virus center in Belem, Brazil. These five viruses, Apeu, Marituba, Murutucu, Caraparu, and Oriboca, were related to one another by either the HI or Neut tests but did not cross react with members of serogroups A or B. A sixth virus, Itaqui, was later added to serogroup C by Causey (4).

The fourth serogroup of arboviruses to be classified differed from the preceding three in that they were related by complement-fixation and not by cross reaction in the HI test. This was the Bunyamwera serogroup reported by Casals and Whitman (11) in 1960. The members of this group were

isolated in widely separated areas: Bunyamwera from Uganda, Africa; Wyeomyia from Columbia, South America; Cache Valley from Utah, U.S.A.; Kairi from Trinidad, West Indies; and Germiston, a possible member, from South Africa. A fifth serogroup which, like Bunyamwera, had to be grouped by CF test because of its poor hemagglutinin properties was the Guama complex made up of Guama and Catu from Brazil, and Bimiti from Trinidad, West Indies (5).

The delineation of these serogroups may have importance in other areas besides taxonomy. Casals (1) showed that prior infection of laboratory animals with virus from one serogroup would give significant protection against infection with another virus of the same serogroup. In human sera from Trinidad he found that sera with neutralizing antibodies against dengue type 2 were capable of protecting mice against infection initiated by interperitoneal inoculation with yellow fever. Stamm and Kissling (12) found that birds that had survived an infection with EEE in the laboratory had a viremia of significantly lower titer and shorter duration when infected with WEE than did controls not previously infected with EEE. In experiments with human volunteers Price (13) showed that an injection of live WN virus followed by one of killed JBE produced higher titers against JBE, SLE, and Murray Valley viruses than did immunization with two injections of killed JBE. Working with serogroup A arboviruses, Hearn (14) in 1963 found that one injection of mice

with an attenuated strain of VEE (9t strain) protected animals against otherwise lethal doses of EEE and Semliki forest viruses.

As immunological characterization of the arboviruses progressed it was shown that the antigenic properties of many of the serotypes previously recognized overlapped to such an extent that questions arose as to their true antigenic distinctness (15). Some of the early work in this field led Hammon (16) to suggest that the arboviruses may have arisen from a common "stem virus" and developed antigenic diversification due to host selection of mutant strains, just as certain growth media will select mutant strains of bacteria.

Arbovirus Strain Variation

The antigenic uniqueness of the arbovirus serotypes has become even less clear in recent years as data have accumulated showing that strains of the same arbovirus may have antigenic differences.

In 1945 Olitsky (17) reported that mice vaccinated with the Rockefeller Institute strain of WEE did not produce protective antibody against infection with other WEE strains. He further showed that this phenomenon was not evident when mice were challenged with the strain used for immunization. He postulated that the antigenic properties of the immunizing strain had changed after numerous mouse passages in the laboratory.

In 1960 Clarke (15) conducted experiments in which she

took serum from animals immunized with one strain of an arbovirus and mixed it with a second strain of the same virus. This second strain would adsorb any heterologous antibodies but leave in the serum antibodies unique to the immunizing strain. By means of this antibody adsorption technique she found that strains of yellow fever virus isolated in the New World lacked an antigen present in African strains of the same virus. She also found that the 17-D strain of yellow fever used for vaccination had acquired an antigen during its process of attenuation that was not present in the parent strain. However, Clarke also found that three strains of WN virus were antigenically identical in spite of their isolation in different countries and from different hosts over a period of fifteen years.

Other workers have shown changes in the infectivity of strains of arboviruses after repeated laboratory passages (18,19), as well as changes in the morphology of tissue culture plaqués formed by arboviruses after a number of culture passages (20,21). These changes have been biological in nature and not associated with changes in the antigenicity of the virus strain.

Evidence that there may exist two biologically distinct sub-populations of virus particles within a given strain population was advanced by Dunayevich and Johnson (22) in 1961 in their studies on the "interference effect" seen with WEE. This "interference effect" was the name given

to the paradoxical survival of young adult mice when given large doses of WEE intercerebrally, while other mice die when given more dilute suspensions of virus. These investigators found that they could select certain clones of virus from tissue culture that would be non-pathogenic for young adult mice and would grow as a pure line of virus. They postulated that within certain strains of WEE virus there were two particle populations, pathogenic and non-pathogenic. Work done at Yale by Henderson (23,24) has shown antigenically distinct sub-populations of virus making up various strains of WEE. These experiments are discussed later.

Strain Variation of Viruses other than Arboviruses

The existence of virus strains showing different antigenic characteristics and containing within themselves distinct sub-populations has been known for some time with the influenza viruses. Friedewald (25) in 1944 using antibody adsorption techniques similar to those used later by Clarke (15) for the arboviruses, showed differences in the antigenic structure of several strains of influenza A virus. In the same year Burnet (25) in Australia showed that a strain of influenza isolated in Melbourne in 1935 had changed antigenic structure after a number of passages in eggs. A few years later Francis (27) in the United States showed much the same thing with a strain passed through tissue culture and eggs, while the same strain passaged in

mice retained its original antigenicity.

Extensive work with antibody adsorption by Hirst (28) and later by Jensen and Francis (29) confirmed the findings of antigenic variation among a large number of strains of influenza A. It was shown that there were at least 18 different antigens distributed in varying proportions among 29 strains of influenza A tested. In 1959 Choppin and Tamm (30) reported that strains of influenza A_2 were composed of two kinds of virus particles which they designated S$\not$ and S-. The S$\not$ were highly sensitive to serum inhibitors, agglutinated red blood cells treated with V. cholerae filtrate, and eluted slowly or not at all from red blood cells. The S- particles, however, showed none of these properties. In later work (31) they succeeded in establishing pure strains of S$\not$ and S- particles and found that the natural strains of influenza A_2 varied widely in their ratio of S$\not$ to S- particles and that the properties of each strain were related to this ratio. Repeated passages of mixtures of S$\not$ and S- particles in chick embryos showed an increase in the S$\not$ to S- ratio regardless of the ratio of the original mixture. Lief (32) in 1964 reported that the S$\not$ and S- particles were antigenically different by complement-fixation.

Studies with poliovirus have shown there were also variations in the antigenic characteristics of strains of this virus. Wenner (33) in 1956 in an analysis of 18

strains of type 2 polio virus found that they could be classified into three groups. There was also some evidence that strains isolated in the same geographic area were more closely related than strains from other parts of the country.

Studies of this type led to investigations of antigenic variation among strains of WEE virus. It may be of interest to consider the history and epidemiology of this virus before relating more specific studies of its antigenic character.

Western Equine Encephalitis Virus

History

Epizootics of a febrile disease of horses, associated with a staggering gait and later, paralysis and death, have been known in the United States since at least 1847. The most widespread epizootic was in 1912 and centered in Nebraska and Kansas where 35,000 horses died (34). The nature of the disease was not known until 1930 when Meyer (35) isolated the virus of WEE from the brains of dead horses during an epizootic in the San Joaquin Valley of California. It was in 1938 that the first human case of WEE was authenticated. This was a fatal case in a 20 month old boy admitted to Fresno County Hospital in California. Howitt (36) succeeded in isolating the virus from the brain stem.

There were numerous epidemics and epizootics in the mid and far west during the 1940's (37-44), but it was not until 1955 that the virus was isolated East of the Appalachians.

This isolation was made by Holden (45) from an English sparrow in New Jersey. He also found neutralizing antibodies in pheasants caged in the same area and concluded from the ages of the birds that WEE had been active in New Jersey in 1952 and 1953.

The first human infection in the East was reported by Paull (46) in 1960. This was a non-fatal case in a 16 year old boy in Pawtucket, Rhode Island. No virus was isolated, and diagnosis was based upon a rise in complement-fixation antibody.

Epidemiology

When the virus of WEE was isolated in 1930 there was no ready explanation for the spread of the disease (34). Three years later Kelser (47) was able to transmit WEE to a horse by means of Aedes aegypti mosquitoes previously fed on infected guinea pigs in the laboratory. A naturally infected arthropod was not found until 1940 when Kitselman and Grundmann (48) isolated WEE from "kissing bugs" (Triatoma sanguisuga) in a pasture in Kansas where fatal equine cases had occurred the year before. In subsequent work, however, they were unable to transmit the virus to laboratory animals by means of T, sanguisuga.

Hammon (49) in 1941 isolated WEE virus from a pool of Culex tarsalis mosquitoes during an epidemic in the Yakima Valley in Washington. The following year Hammon (50) reported five more isolations from C. tarsalis in this Yakima

Valley, and in 1945 he reported 41 isolations of WEE from C. tarsalis during a 1942 epidemic in Washington (39).

There have been numerous other isolations of WEE virus from Culex tarsalis (39,51-54), and most workers accept this mosquito as the principle vector of WEE virus in the West (16). Reaves (55) has found that the virtual disappearance of WEE from a formerly endemic area was coincident with a great decline in the C. tarsalis population of the area after the introduction of DDT.

Birds, and in particular domestic fowl, have often been shown to be infected with WEE virus. Hammon (56) in 1942 found that 50% of domestic fowl and 17% of wild birds in Washington had neutralizing antibodies. In an endemic area of California he also found 26% of chickens to have neutralizing antibodies (39). In addition, Burroughs (51) found 13% of chickens tested in Minnesota had antibodies. Other birds found to have a high percentage of sera-containing WEE antibody include pheasants (45), ducks (57), and pigeons (58). This evidence of avian infection with WEE lends more support to the implication of C. tarsalis as a vector, since this mosquito feeds heavily on birds, particularly domestic fowl (39,51,59). It has also been shown that the viremia level necessary to infect C. tarsalis is lower than for most other mosquitoes (60).

From these data emerges the picture of WEE as a disease having a mosquito-bird-mosquito cycle with man or horse as

an accidental host (53). Hammon and Reeves (42,61) point out that while this cycle may function in this manner in the irrigated valleys of the far west there may be a different cycle in the dry, open farmlands of the midwest. As evidence they note the low rate of infection found in fowl in epidemic areas of Texas, Colorado, and Nebraska. Although the number of isolations in C. tarsalis far exceed those for any other mosquito the following have all been considered as vectors: Aedes dorsalis, Aedes vexens, Aedes aegypti, Aedes triseriatus, Aedes atropalpus, Aedes nigromaculis, Aedes sollicitans, Culex stigmatosa, Culex pipens, Culex restuans, Culesita inornata, Psorphora discolor, Orthopodomyia signifera, and Anopheles maculipennis freeborni (42,61,62).

Sulkin (38) in 1945 reported isolating WEE from chicken mites (Dermanyssus gallinae) in Texas from a farm where an equine case had occurred. Although other workers have tested thousands of chicken mites, no other isolation has been reported (39,42,51,61). There is evidence that in some exceptional cases bird to bird transmission may occur without arthropod intervention. Reeves (63) found that wild birds placed in a mosquito proof aviary with artificially infected birds developed neutralizing antibodies to WEE. Burton (57) found that ducks given large doses of WEE orally developed a viremia and neutralizing antibodies. Winn and Palmer (64) report that pigeons

inoculated with WEE virus were found to have virus in their crop 18 to 96 hours after inoculation, although virus could not be recovered from their feces. Since young birds are fed on food regurgitated from their parents' crop they suggest this may be a route of infection for the young.

There is still a considerable problem as to how WEE is maintained in an area over the winter. Blackmore and Winn (54) suggest three possibilities: 1) migrating birds bring the virus north each spring, 2) arthropods other than mosquitoes harbor the virus in the winter and infect birds in the spring, and 3) infected mosquitoes hibernate over winter and infect birds the following spring.

There has been only one isolation of WEE from hibernating mosquitoes (54). Reeves (52) was unable to recover WEE from C. tarsalis in California during a period from November 15 to January 15 during a five year study, although he did isolate it at all other times during the year.

To investigate the theory that migrating birds reintroduce the virus to endemic areas each spring, Kissling (64) tried to isolate WEE virus from birds wintering in Louisiana and Florida. He found no virus in 1,503 birds, although some had antibodies.

A possible vector for carrying WEE over the winter is the wood tick (Dermacentor andersoni). Syverton and Berry (66) were able to transmit WEE to rodents in the laboratory with this anthropod. They also found that the

offspring of infected ticks were infected and capable of transmitting the infection (67). There has, however, been no isolation of WEE from Dermacentor andersoni in nature.

Thomas and Eklund (68) in 1960 showed experimentally that artificially inoculated garter snakes could maintain infections of WEE through the winter and develop a viremia of sufficient titer after hibernation to infect mosquitoes. Again it must be stated that there has been no isolation in nature from these proposed hosts. Reeves (60) in 1963 found no evidence of C. tarsalis having fed on reptiles, rodents, or amphibians in a study of blood meals with precipitating antisera.

In summary it can be said that WEE appears to be transmitted primarily by Culex tarsalis in a bird-mosquito-bird cycle (at least in the far west), that this cycle may be interrupted by transmission to another animal, and that it is not known whether the virus is maintained over the winter in endemic areas.

Strain Variation in WEE

The work of Olitsky (17) showing antigenic variation among strains of WEE has already been mentioned, as has the biological variation shown in sub-populations of WEE by Dunayevich and Johnson (22). In 1963 Karabatsos, et al, (69) showed marked differences between laboratory strains and recent isolates of WEE. All of the laboratory strains had undergone many mouse passages and were antigenically

indistinguishable by HI, Neut or complement-fixation tests. The recent isolates were antigenically different from the high passage strains. More recent work by Henderson (23,24) has shown the existence of antigenically distinct sub-populations of virus within various strains of WEE. Henderson also reacted 100 strains of WEE virus against antisera prepared against antigenically different plaques derived from one WEE strain and was able to group strains into three categories. Fifty three strains were neutralized by all three of the plaque antisera. These were called "aphasic strains." Thirteen strains were called "phase I," and 34 strains were designated "phase II." Furthermore he found that strains from the western United States were aphasic or phase I, while phase II strains were found on the Atlantic and Gulf coasts, with the exception of one phase II strain found in Colorado. Phase I and phase II virus strains were found to convert to aphasic after a number of passages in mouse brain or rhesus monkey kidney culture, but aphasic strains were stable. No phase change was noted after passage in chick embryo tissue culture.

MATERIALS AND METHODS

Collection of Sera

In the summer of 1962 a serum survey of United States military recruits was conducted under the direction of

Dr. J. R. Paul of the Department of Epidemiology and Public Health, Yale University School of Medicine, and under the auspices of the Armed Forces Epidemiological Board and the WHO Reference Serum Bank (70). The United States was divided into ninety divisions based on ecological considerations (Figure 1). An effort was made to secure sera from fifty recruits from each division with an equal number of recruits from each division with an equal number of recruits from rural (population under 2500) and urban communities. The blood samples were collected from recruits at seven training centers, about 500 samples from each center.

All recruits filled out a questionnaire listing their age, ethnic group, birth place, residences, previous occupation, poliomyelitis vaccinations, and exposure to animals. A sample questionnaire is shown in Figure 2. Candidates to be bled were selected on the basis of their place and duration of residence in one area. The majority had lived within a radius of fifty miles of their birth place all their lives, and a large proportion of the rest had lived within a similar radius for over half their lives. Geographic distribution of sample is shown in Figure 3.

Blood was drawn prior to any immunizations. Disposable sterile needles fitted to 20 ml vacutainers were used. Blood was allowed to clot for twelve hours at $4^{\circ}C$, then it was centrifuged for 15 minutes at 2000 rpm. The serum was pipetted into test tubes and recentrifuged to remove the

few red cells usually transferred in the pipetting. The clear serum was frozen, packed in dry ice and flown to Yale University. Approximately 3200 sera were collected in this manner, and the geographical quotas were well realized.

Since this survey represents only males from 17 to 24 years of age who met the Armed Forces physical requirements, it must be considered only a sample of the young, healthy, male population of the United States. It is not at all a representation of the population as a whole.

Virus Strains

Two strains of WEE virus, the McMillan and Alabama strains, were used to prepare the antigens for these experiments. The McMillan strain was isolated in 1941 from a fatal human case in Winnipeg, Manatoba (71). The fourteenth mouse passage of the virus was used. Henderson (24) has shown that this virus is an aphasic form of WEE.

The Alabama strain was isolated in Alabama in 1957. The fifth mouse passage was used in these experiments. The virus was found by Henderson (24) to be a phase II type.

The Hemagglutination Inhibition Test

Antigen Preparation --- Antigens for HI tests were prepared according to the acetone-sucrose extraction method from brains of suckling mice as outlined by Clarke and Casals (72).

Extraction of Sera --- Work at the Yale virus laboratory has shown that the kaolin method of extraction given by Clarke and Casals (72) resulted in a number of false positive reactions in the HI test with the recruit sera. Because of this the more tedious, but more reliable, acetone precipitation method was used (72). For this the sera were diluted 1:10 and 12 volumes of chilled acetone added. This mixture was allowed to stand for 5 minutes and then was centrifuged in the cold at 1,800 rpm. The supernatant was discarded and 12 more volumes of chilled acetone were added, and again the mixture was centrifuged. The supernatant was again discarded, and the sediment was dried for 30 minutes in a desicator attached to a vacuum pump. The dried sediment was rehydrated with 2 ml of borate saline, pH 9, and allowed to stand overnight in the refrigerator. The final treated sera were stored at -20°C until tested for HI antibodies.

Preparation of Chick Erythrocytes --- Erythrocytes for HA and HI tests were obtained from 1-day-old chicks, which were bled by cardiac puncture. Blood was diluted 1:4 in Alsever's solution and stored in the refrigerator. Chick erythrocytes were washed three times in phosphate buffered saline (PBS) and resuspended to 10%. Aliquots of the stock 10% suspension were diluted twenty times with pH adjusting buffers for use in the HA or HI tests.

Hemagglutination Titrations --- The HA test was

performed to establish the titer of the antigen for subsequent use in the HI test, and to determine the optimal pH for each of the antigens. Clear plastic "agglutination trays" were used, each containing eighty shallow spherical wells about 3/4" in diameter. Tests were performed according to the method described by Clarke and Casals (72). Virus antigens were tested at five pH values: 6.2, 6.3, 6.4, 6.6, and 7.0.

Results of each titration were read after one hour. Complete agglutination appeared as a thin, transparent shield of red cells over the whole surface of the wall. Lack of agglutination appeared as a red button in the center of the well. Virus titer was read as the last dilution giving complete hemagglutination.

Hemagglutination-Inhibition Titrations --- HA antigen was diluted so as to give 4 to 8 units contained in 0.2 ml, based on the titer determined by the HA test. This dilution was calculated by dividing the HA titer by sixteen. Each serum used had been found to inhibit agglutination by WEE at 1:10 in an initial screening. Two-tenths ml bovine albumin-borate saline (BA-BS) was placed in each of five wells in the agglutination trays. Then, 0.2 ml of each serum at a 1:10 dilution was carried through five serial 2-fold dilutions (1:20 to 1:360). Two-tenths ml of the antigen containing 4 to 8 HA units was added to each well, and the mixture was left for one hour at room temperature. Then

0.4 ml of a 1:200 chick erythrocyte suspension, made up in the appropriate pH buffer, was added to each well. After standing for one hour at room temperature, the hemagglutination patterns were read. The titer of the serum was taken as that dilution which showed complete HI.

Controls consisted of 0.4 ml of cells mixed with 0.4 ml of BA-BS, and 0.4 ml of cells mixed with 0.2 cc of antigen and 0.2 ml of BA-BS.

The Neutralization Test

Preparation of Virus --- Suckling mice were intracerebrally inoculated and the brains harvested and pooled. The group of brains were homogenized with sufficient PBS added to make a 10% suspension. The homogenate was centrifuged at 10,000 rpm for thirty minutes in the cold. The supernatant was pipetted into Wasserman tubes in 0.25 ml aliquots. Then, 0.25 ml of 10% bovine albumin solution was added to each tube. All tubes were stored at -70°C.

Preparation of Tissue Culture --- Chick embryo fibroblast tissue cultures were used as described by Henderson and Taylor (73). Nine-day-old White Rock chick embryos were decapitated and minced with scissors. The minced tissue was washed with cold phosphate buffer and the cells dispersed with trypsin. The cell suspension was filtered through four layers of gauze. The cells in the filtrate were then washed three times in cold buffered saline, packed by low speed centrifugation, and resuspended at a

concentration of about 1:400 in Hanks' growth medium. Three-ounce prescription bottles were seeded with 8-10 ml from this suspension and incubated in a horizontal stationary position at 37°C for three days prior to use.

The Test --- Log dilutions of each virus were mixed with equal amounts of 1:8 dilution serum. The mixture was allowed to stand at room temperature for one hour. Then 0.2 ml of each virus-serum mixture was added to a tissue culture bottle (after discarding the growth medium). The inoculum was spread evenly over the cell sheet, and bottles were incubated in a horizontal position with the cell sheet down for one hour at 37°C.

Each cell sheet was overlaid with an agar-medium. When the agar had cooled, all bottles were inverted and placed in the incubator at 37°C. Plaques appeared in 2-3 days. At the end of two days the number of plaques seen in each virus dilution was compared to control bottles of the same dilution without serum added. When comparing Neut titers obtained with test sera against the two virus strains, a difference of 1.5 logs was considered to be significant.

CHEMICAL REAGENTS

Borate-saline, pH 9.0 --- This solution is 0.05 M sodium borate and 0.12 M NaCl. It is made by diluting 80 ml of 1.5 M NaCl, 100 ml of 0.5 M Na borate and 24 ml of 1.0 M NaOH up to one liter with distilled water.

Bovine albumin-borate saline (BA-BS) --- A 4% solution of bovine albumin is made with borate saline and the pH is adjusted to 9.0 with 2 M NaOH.

Buffers for HA and HI tests --- These buffers were prepared from mixtures of 0.15 M NaCl-0.2 M Na_2HPO_4 and 0.15 M NaCl-0.2 M NaH_2PO_4. Clarke and Casals (72) list the proportions needed for making buffers which give pH values from 5.75 to 7.4 when mixed with equal volumes of borate-saline, pH 9.0.

Hanks Growth Medium --- To make one liter the following proportions were used: 769 ml water; 100 ml of Hanks' 10x concentrated; 100 ml of lactalbumin hydrolysate (5%); 20 ml of calf serum (sterile); 7 ml of $NaHCO_3$ (7.5%); 4 ml of phenol red (0.5%); 1 ml of penicillin (200,000 u/ml) and 0.4 ml of streptomycin (500,000 mg/ml).

Agar preparation --- 2.4 gms of Difco agar were used for each for each 90 ml of water. The agar was added to the water and melted in a bath of boiling water. Once dissolved, the agar was autoclaved at 15 lbs for 15 minutes and temporarily stored in a 56°C water bath.

Agar overlay media --- The following amounts were used for each 90 ml of medium (enough for 16 3-ounce bottles): 60 ml of sterile, demineralized water; 18 ml of Earle's saline, 10x concentrated; 6 ml of yeast extract (1% aqueous); 6 ml of lactalbumin hydrolysate (5% aqueous); 3.6 ml calf serum; 5.4 ml of $NaHCO_3$ (7.5%); 3.0 ml neutral red (1:1000);

0.2 ml of penicillin (2000,000 u/ml), and 0.1 ml of streptomycin (500,000 mg/ml). These were mixed thoroughly with an equal volume of melted agar just before use.

RESULTS

Hemagglutination Inhibition Tests

Of the 3200 sera screened at a 1:10 dilution for HI antibodies to WEE virus, 41 were found to be positive. The screening was done with a phase II virus. Thirty-six of the 41 positives were available for titration against an aphasic (McMillan) and a phase II (Alabama) strain of WEE virus.

Twenty-one showed no HI antibodies at a 1:20 dilution when tested against the aphasic strain and a titer of only 1:10 against the phase II virus. Results of these titrations are shown in Table 1. It will be noted that the residences of the recruits from whom these sera were obtained were largely confined to the East and the Midwest. The other 15 sera tested had a titer of 1:20 or greater against one or both of the test viruses. Results of titration of sera are shown in Table 2. These samples were from residents living in the West and the Far West.

Two of these sera showed a four-fold difference between the titers when tested against the two virus strains. These sera, #894 and #2323, were from recruits living in Fresno County, California. It is of interest that #894 showed a

four-fold increase in antibodies to phase II virus but not aphasic virus, while #2323 showed just the opposite. A third serum, #1362, gave a relatively low titer (1:40) against the phase II virus and a titer of less than 1:20 against the aphasic strain.

It is also of some interest that 4 of these 15 sera were from recruits living in Nebraska.

Neutralization Tests

Of the 41 sera which were positive for WEE by HI screening, 29 were available for tissue culture neutralization tests with the aphasic and phase II strains of the virus. The plaque-forming unit (PFU) log reductions by these sera against each virus are shown in Table 3. Nine of these sera (#698, #887, #1047, #1285, #1359, #1397, #2323, #2403, and #2517) showed a significant neutralization titer when using one or both of the virus strains. All nine showed significant titers against the aphasic virus, but only four had significant titers against both viruses.

When sera #698 and #1285 were tested in the plaque reduction neutralization test against the two virus strains, there was a 1.5 or more log difference in titer. There were differences of lesser magnitude noted in 6 other sera (#1047, #1359, #2323, #2403, #2517). In two of these the greater titer was against the phase II antigen. In four, however, the higher titer was against the aphasic antigen. The two

sera which had a greater neutralizing capacity for the phase II strain of WEE were from recruits living in Nebraska.

Variation between HI and Neut Test Results

In Table 4 are compared data obtained from sera on which both HI and Neut tests were run and which showed significant differences in titers against the two virus strains measured by either test. It will be noted that serum #694 was of borderline significance by both tests. The HI and Neut differences were not always of the same magnitude. Serum #1285 showed a marked difference when reacted against the two phase viruses by Neut, but no difference by HI test. Serum #2323 showed a six-fold difference between the two phase viruses by HI test, but a log PFU reduction difference of only 1.2.

DISCUSSION

Thirty-eight sera were tested in this study and of these, seventeen showed significant antibody levels by the HI or Neut test. Even though this is far too meagre a sample to provide conclusive data concerning antibody type variation in WEE, certain aspects of the study deserve comment.

One of the most striking findings was the geographic division between the viruses which had an HI titer of only

TABLES AND FIGURES

2. Twenty-one sera had an HI titer of only 1:10 and sixteen of these were tested by Neut and found to have no titer. Most of these sera were from donors living in the eastern U. S. The sera showing significant HI and/or Neut titers were from donors in the mid- and far west.

3. Eight sera showed a significant difference in antibody levels to the two phases of WEE virus by one or both of the tests. The Neut test gave better differentiation between the two strains.

4. Antibody type, resulting from infection by aphasic or phase II viruses and demonstrated by the Neut test, seems to have a similar geographic distribution as that of the different antigenic phases of WEE virus. Results with the HI test are ambiguous on this point.

In this study the sera which showed a higher level of antibodies to the aphasic strain of WEE were from recruits living in North Dakota and South Dakota, California, Oklahoma, and Washington. This distribution corresponds to the areas in which aphasic virus has been isolated. The sera which had a higher Neut titer to the phase II strain were both from recruits living in Nebraska. Although no phase II virus has been isolated in this state it has been isolated in the neighboring state of Colorado.

The two sera which showed antibody strain variation by the HI test were both from residents of Fresno, California. One showed a higher HI titer to the aphasic strain as might be predicted, but the other (#894) showed a higher titer against the phase II form.

A phase II strain of WEE has never been isolated in California, and the high titer of serum #894 to phase II is puzzling. According to their donor's questionnaire he lived for two years (1957-59) in Maine. Since this is the region where phase II isolates of WEE have been made, it may be that he acquired his infection there.

SUMMARY

1. Thirty-eight sera which were known to have HI antibodies to WEE virus at 1:10 dilution were tested by HI and Neut tests against two strains of WEE virus representing different antigenic phases.

1:10, and those which had a more significant antibody level. Sixteen of the twenty-one sera with an HI titer of 1:10 were from recruits living east of the Missouri River, and eight of them were from recruits living in Atlantic coastal states. In contrast the 17 sera with more significant HI or Neut titers were from recruits living west of the Missouri River with only one exception; this exception, from Iowa, was of borderline significance.

Sixteen of the twenty-one sera with an HI titer of 1:10 were tested by Neut, and none were found to show significant neutralization, while all of the sera with higher HI titers that were tested by Neut were found to have significant neutralization titers.

The failure of the sera with HI titer of 1:10 to neutralize WEE, together with their geographic distribution, suggest that their donors may have been infected with another arbovirus related to WEE, and their low HI titer represents a cross reaction. The only other serogroup A virus known to be active in the eastern United States is EEE. These same sera were screened for HI antibodies to EEE in another study, and none of them were positive.

The low HI titer of these sera may also be the result of non-specific inhibitors or very old WEE infections, but in either case it would be hard toexplain why they were not randomly distributed across the country instead of being localized in the East.

Of the two tests the Neut test seemed better able to pick up differences in antibodies to the two strains. Of the five sera showing borderline or significant antibody variation by Neut, there are HI titers for only four. In only one case does the HI test show a significant degree of antibody variation.

It is interesting to speculate why a few sera showed antibody variation, and the rest do not. It may be that sera which do not show antibody strain variation are from individuals who have been infected more than once with WEE, the second infection causing an amnestic response with increased titer to all the antigens of the virus. Some of these sera may also represent old infections with WEE in which the originally high titer to one strain has declined. Other factors such as the previous experience of the infected individual with other serogroup A viruses, the amount of the inoculum, the severity of the infection, all may play a part in determining whether the serum antibodies will show strain specificity.

From the data here, it might have been concluded that antibody strain variation can be determined in sera of some individuals previously infected with WEE, but such antibody variation is not always found.

In Henderson's study (24) the phase II of WEE virus was found on the east coast, while the phase I and aphasic forms of the virus were isolated in the mid- and far west.

TABLE 1

Sera Inhibiting Hemagglutination at a 1:10 Dilution Only Using Alabama (Phase II) Virus as HA Antigen

Serum	Residence of Recruit		
	City	County	State
18	Elmhurst	Queens	N.Y.
71	Union City	Hudson	N.J.
94	Woodsville	Grafton	N.H.
100	Elizabeth	Union	N.J.
1078	Washington	Franklin	Mo.
1106	Marlow	Stevens	Okla.
1108	Britt	Hancock	Iowa
1121	Santa Fe	Santa Fe	N.M.
1154	Brownfield	Terry	Texas
1157	Springfield	Greene	Mo.
1177	Stewartville	Olmstead	Minn.
1252	No. Vernon	Jennings	Ind.
1293	Bettendorf	Scott	Iowa
1313	Hersey	St. Croix	Wis.
1376	Ardmore	Carter	Okla.
1492	Hamburg	Calhoun	Ill.
1503	Martin	Bennet	S.D.
1864	Cadyville	Clinton	N.Y.
1865	Coward	Florence	S.C.
1866	Belvedere	Aiken	S.C.
1868	Easley	Pickens	S.C.

TABLE 2

Sera with HI Titers Greater Than 1:10 Using Two Antigenically Different WEE Strains (McMillan and Alabama)

Serum	WEE Virus Strains		Residence of Recruit		
	McM	Ala	City	County	State
694	20*	10*	Farmington	San Juan	N.M.
894	40	160	Fresno	Fresno	Calif.
1047	80	40	Rapid City	Pennington	S.D.
1052	20	20	Grant	Perkins	Nebr.
1285	40	40	Alva	Woods	Okla.
1324	80	80	Avendale	Manicopa	Ariz.
1330	20	10	Stockton	Rooks	Kan.
1359	80	160	Lindsay	Platte	Nebr.
1362	<20	40	Cheyenne	Laramie	Wyom.
1371	<20	20	Davenport	Scott	Iowa
1397	160	160	Sidney	Cheyenne	Nebr.
1455	-	160	Kimball	Kimball	Nebr.
2323	320	40	Firebaugh	Fresno	Calif.
2403	40	20	Mt. Vernon	Skagit	Wash.
2517	40	20	Grafton	Walsh	N.D.

* Reciprocal of highest dilution of serum giving HI

TABLE 3

Neutralization Test Titers Using Antigenically Different WEE Strains

Serum	WEE Virus Strains		Residence of Recruit		
	McM	Ala	City	County	State
100	0.4*	0*	Elizabeth	Union	N.J.
694	1.4	1.3	Farmington	San Juan	N.M.
698	4.1	1.5	Carrington	Foster	N.D.
887	1.8	1.2	Whittier	Los Angeles	Calif.
1047	1.7	0.9	Rapid City	Pennington	S.D.
1052	0.4	0	Grant	Perkins	Nebr.
1078	0.4	0	Washington	Franklin	Mo.
1106	0.2	0.5	Marlow	Stevens	Okla.
1108	0	0.1	Britt	Hancock	Iowa
1121	0.1	0.1	Santa Fe	Santa Fe	N.M.
1157	0.4	0.1	Springfield	Greene	Mo.
1177	0.4	0	Stewartville	Olmstead	Minn.
1252	0.1	0	No. Vernon	Jennings	Ind.
1285	2.7	1.0	Alva	Woods	Okla.
1293	0.1	0.6	Bettendorf	Scott	Iowa
1359	2.2	2.9	Lindsay	Platte	Nebr.
1362	-	0.2	Cheyenne	Laramie	Wyom.
1371	0.2	0.2	Davenport	Scott	Iowa
1376	-	0	Ardmore	Carter	Okla.
1397	2.5	3.2	Sidney	Cheyenne	Nebr.
1492	-	0	Hamburg	Calhoun	Ill.
1503	0.6	0.7	Martin	Bennet	S.D.
1864	0.4	0.1	Cadyville	Clinton	N.Y.
1865	0.4	0.1	Coward	Florence	S.C.
1866	0.2	0.2	Belvedere	Aiken	S.C.
1868	0.4	0	Easley	Pickens	S.C.
2323	3.0	1.8	Firebaugh	Fresno	Calif.
2403	2.5	1.4	Mt. Vernon	Skagit	Wash.
2517	2.5	1.2	Grafton	Walsh	N.D.

* Log_{10} PFU Reduction

TABLE 4

Summary of Representative Results of Differences in Antibody Levels Against Two Antigenically Distinct WEE Strains Using the HI and Neut Tests

Serum	HI		Neut	
No.	McM	Ala	McM	Ala
694	20*	10*	1.4**	1.3**
1047	80	40	1.7	0.9
1285	40	40	2.7	1.0
1359	80	160	2.2	2.9
1397	160	160	2.5	3.2
2323	320	40	3.0	1.8
2403	40	20	2.5	1.4
2517	40	20	2.5	1.2

* Reciprocal of highest dilution of serum giving HI

** Log_{10} PFU Reduction

LEGEND FOR FIGURE 1

Map of the United States showing the 90 ecological divisions on which the selection of the recruit samples was based.

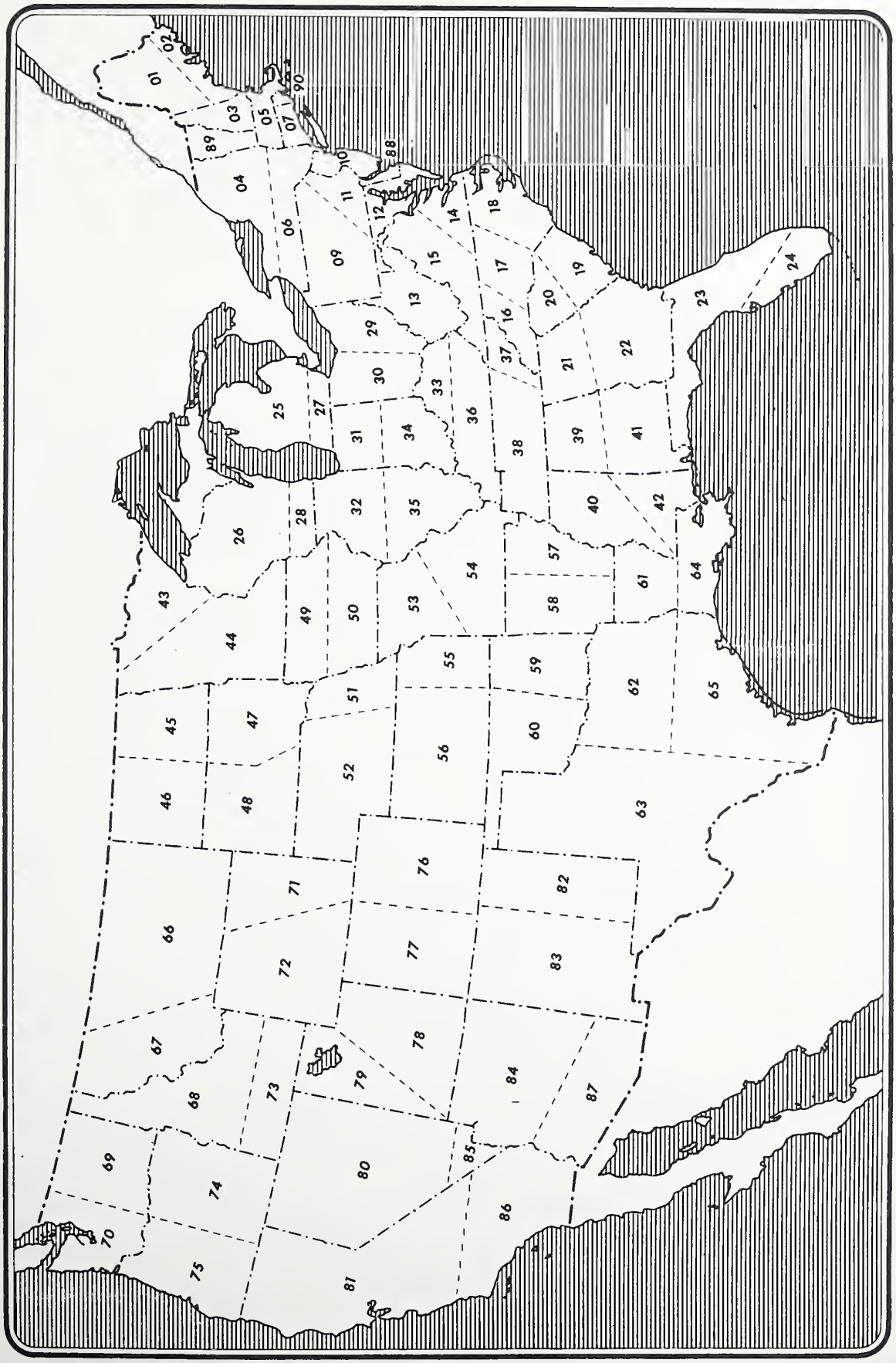
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Figure 1

LEGEND FOR FIGURE 2

A questionnaire of the kind filled out by each recruit prior to his inclusion in the serum survey.

ARMED FORCES SERUM SURVEY
TRAINEE'S REPORT

Section A to be filled in by physician

Ethnic data:

Specimen Code No.: .....

Place: .....

Specimen for test obtained by: ..... Date: .....

Do not write above this line.

Name: ..... Age: ..... Birthdate: .....
(Last) (First) (Middle Initial) Day Month Year

Armed Forces Serial No.: ..... Height: ..... Weight: .....

Present Hometown: .... Approximate population size: .....
City (Place) State

Previous Residence 1) .... Approximate date moved from: .....
City (Place) State

Previous Residence 2) .... Approximate date moved from: .....
City (Place) State

Birthplace: ....
City (Place) State or Country

Former Occupation(s): .....

No. of Brothers and Sisters (total): ..... No. older Brothers: ..... No. older Sisters: .....

Have you had a family Dog? Yes ..... No ..... Cat? Yes ..... No ..... Farm Animals? Yes ..... No .....

Have you been vaccinated against polio? Yes ..... No ..... Unknown ..... (If yes, how many shots?) .....

Have you taken polio vaccine by mouth? Yes ..... No .....

Do not write below this line. For Office use only.

Code No.: ☐☐☐☐

Induct. Center: ☐

Age: ☐☐

Birthplace: ☐☐

Ethnic Gp. ☐

Home State: ☐☐

Prev. States: ☐☐

Urban-rural: ☐

Prev. Res. (1): ☐☐

Mobile: ☐

Occupations: ☐☐

No. of siblings: ☐☐

Sibling position: ☐

Expos. to animals: ☐

Soc.-Econ. Gp. ☐

Vaccination against polio: ☐

No. inoc. ☐

Oral: ☐

Figure 2

LEGEND FOR FIGURE 3

Map of the United States showing place of residence of each of the recruits included in the survey. Each dot represents one recruit.

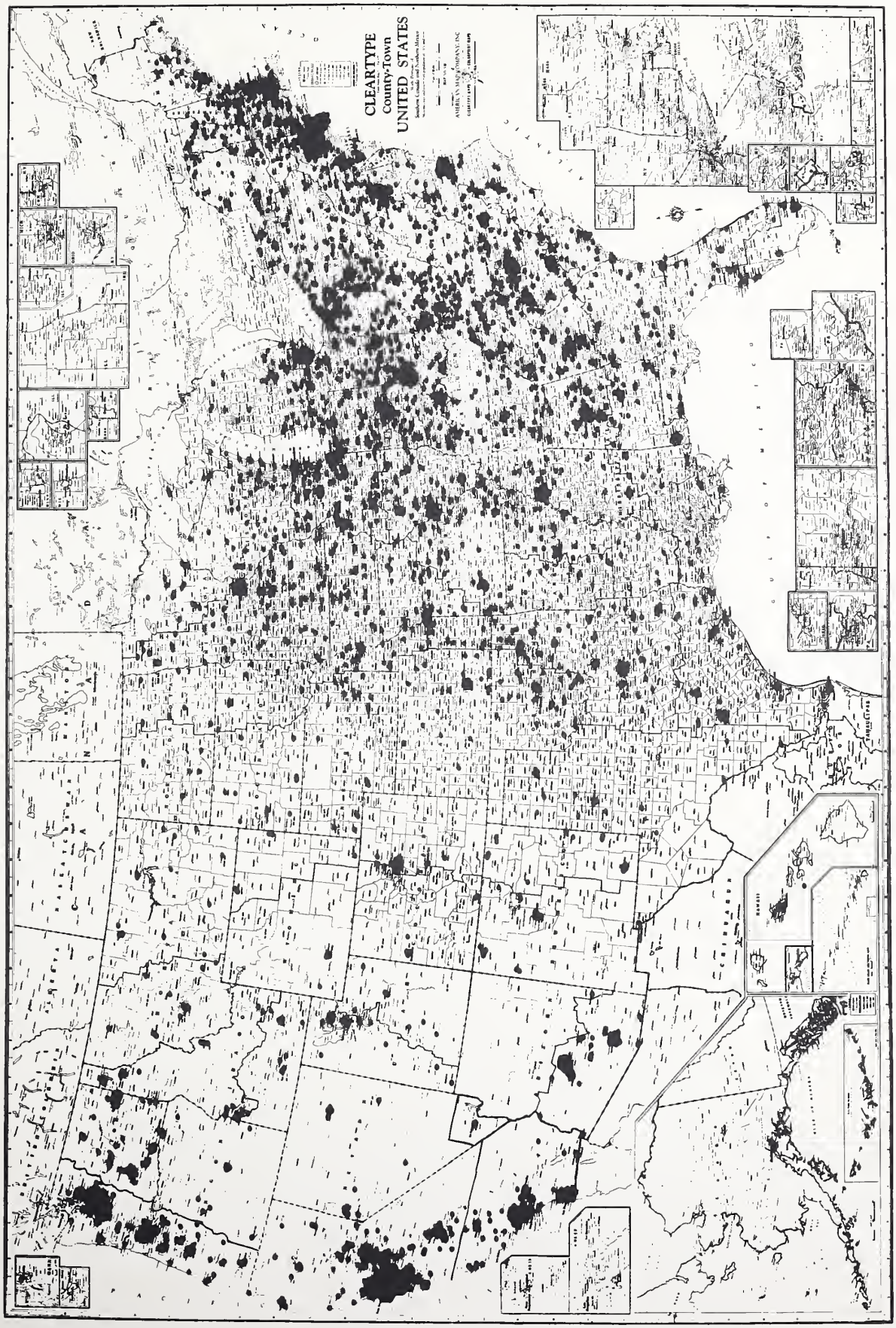
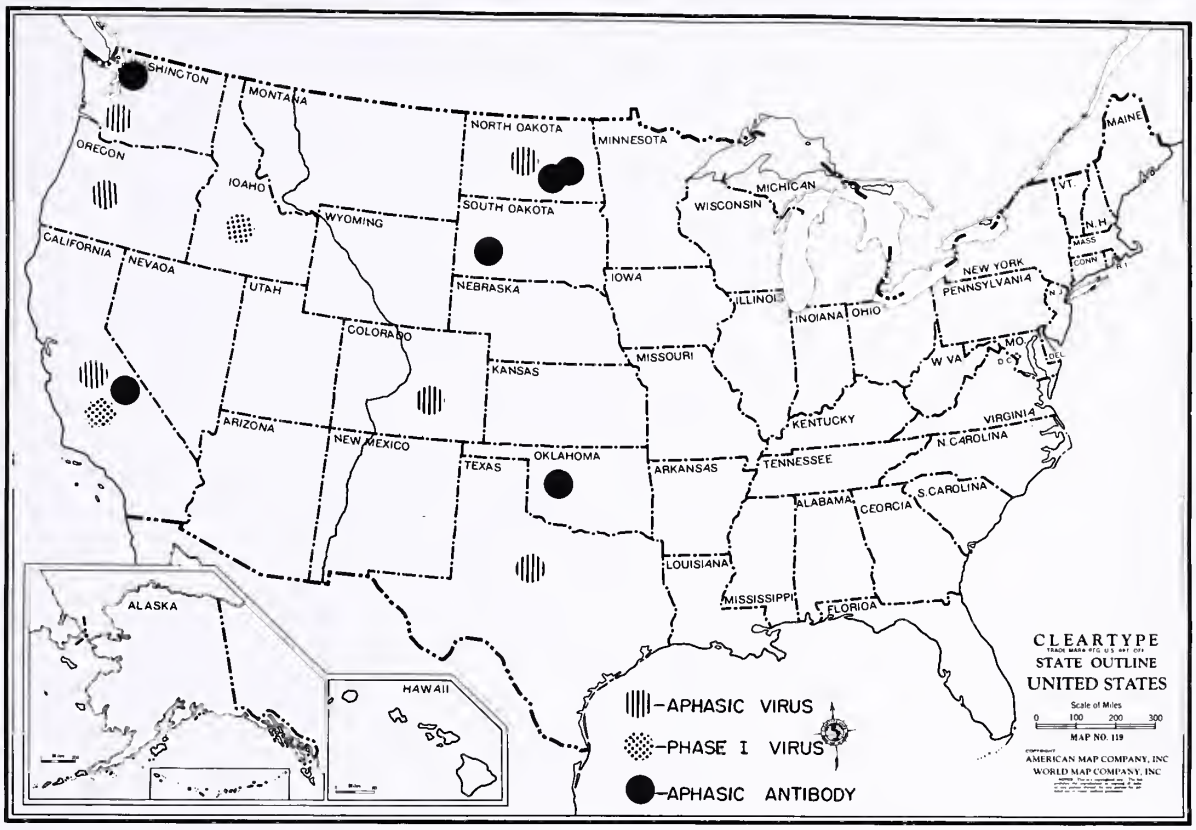


Figure 3

LEGEND FOR FIGURE 4

Top - Map showing the places where aphasic strains of WEE have been isolated, and the residences of the recruits with aphasic neutralizing antibody.

Bottom - Map showing the places where phase II strains of WEE have been isolated, and the residences of the recruits with phase II neutralizing antibody.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MASS.
CONN.
R.I.
CALIFORNIA
NEVAOA
UTAH
NEBRASKA
IOWA
ILLINOI
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OHIO
NEW YORK
PENNSYLVANIA
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MISSOURI
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MD.
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NEW MEXICO
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N.CAROLINA
TEXAS
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ARKANSAS
TENNESSEE
ALABAMA
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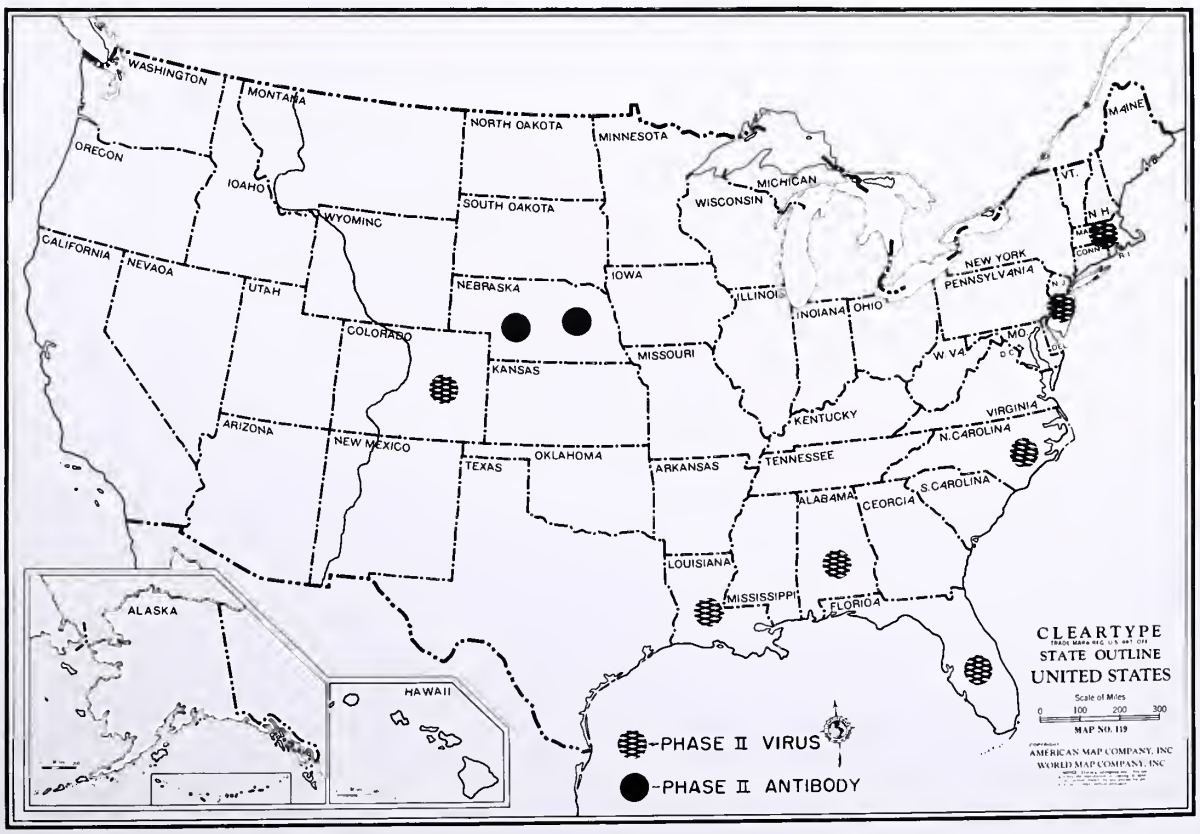
AMERICAN MAP COMPANY, INC
WORLD MAP COMPANY, INC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STATE OUTLINE
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Figure 4

BIBLIOGRAPHY

1. Casals, J., The Arthropod-borne group of animal viruses, Tr. N.Y. Acad. Sc. (ser 2), 19, 219-235, 1957.

2. Casals, J., and Reeves, W.C., Arthropod-borne animal viruses, in Viral and Rickettsial Infections of Man, Rivers, T.M., and Horsfall, F.L. (ed), J.B. Lippincott Co., Philadelphia, 1959.

3. Taylor, R.M., Scherer, W.F., and Work, T.H. (ed), Catalogue of arthropod-borne viruses, published by sub-committee on information exchange of the American Committee on Arthropod-Borne Viruses.

4. Shope, R.E., Causey, C.E., and Causey, O.R., Itaqui virus: A new member of arthropod-borne group C, Am. J. Trop. Med. and Hyg., 10, 264-265, 1961.

5. Whitman, L., and Casals, J., The Guama group: A new serological group of hitherto undiscovered viruses. Immunological Studies, Am. J. Trop. Med. and Hyg., 10, 259-263, 1961.

6. Casals, J., and Whitman, L., Group C: A new serological group of hitherto undescribed arthropod-borne viruses. Immunological Studies, Am. J. Trop. Med. and Hyg., 10, 250-258, 1961.

7. Smithburn, K.C., Differentiation of the west Nile virus from the viruses of St. Louis and Japanese B encephalitis, J. Immunol., 44, 25-31, 1942.

8. Havens, W.P., Jr., Watson, D.W., Green, R.H., Lavin, G.I., and Smadel, J.E., Complement fixation with the neurotropic viruses, J. Exper. Med., 77, 139-153, 1943.

9. Casals, J., Immunological relationships among CNS viruses, J. Exper. Med. 79, 341-359, 1944.

10. Casals, J., and Brown, L.V., Hemagglutination with arthropod-borne viruses, J. Exper. Med. 99, 429-449, 1954.

11. Casals, J., and Whitman, L., A new antigenic group of arthropod-borne viruses: The Bunyamwere group, Am. J. Trop. Med. and Hyg., 9, 73-77, 1960.

12. Stamm, D.D., and Kissling, R.E., The influence of reciprocal immunity on eastern and western equine encephalomyelitis infection in horses and English sparrows, J. Immunol., 79, 342-347, 1957.

13. Price, W.H., Studies on the immunological overlap among certain arthropod-borne viruses, Proc. Nat. Acad. Sc. 43, 115-121, 1957.

14. Hearn, H.J., Jr., Cross protection in animals infected with group A arthropod-borne viruses, J. Immunol., 90, 720-724, 1963.

15. Clarke, D.H., Antigenic analysis of certain group B arthropod-borne viruses by antibody absorption, J. Exper. Med., 111, 21-32, 1960.

16. Hammon, W. McD., The arthropod-borne virus encephalitides, Am. J. Trop. Med., 28, 515-525, 1948.

17. Olitsky, P.K., Morgan, I.M., and Schlesinger, R.W., Vaccination with various western equine encephalitis viruses; comparison as antigens and as test inocula, Proc. Soc. Exper. Biol. and Med., 59, 93-97, 1945.

18. Rohitayodhin, S. and Hammon, W. McD., Studies on Japanese B encephalitis virus vaccine from tissue culture: II Development of an attenuated strain of virus, J. Immunol. 89, 589-597, 1962.

19. Hearn, H.J., Jr., Differences among virus populations recovered from mice vaccinated with an attenuated strain of Venezuelan equine encephalomyelitis virus, J. Immunol., 87, 573-577, 1961.

20. Marshall, I.D., Serivani, R.P., and Reeves, W.C., Variation in the size of plaques produced in tissue culture by strains of western equine encephalitis virus, Amer. J. Hyg., 76, 216-224, 1962.

21. Ushijima, R.N., Hill, D.W., Dolana, G.H., and Gebhardt, L.P., Plaque mutants of western equine encephalitis virus, Virology, 17, 356-357, 1962.

22. Dunayevich, M., Johnson, H.N., and Burleson, W., Selection of a clone of western equine virus which is not pathogenic for young adult mice, Virology, 15, 295-298, 1961.

23. Henderson, J.R., Virus sub-populations comprising various strains of western equine encephalitis virus, Fed. Proc. (abstract), 21,460, 1962.

24. Henderson, J.R., Immunological characteristics of western equine encephalitis virus strains, J. Immunol., In Press.

25. Friedewald, W.F., Qualitative differences in the antigenic composition of influenza A virus strains, J. Exper. Med. 79, 633-647, 1944.

26. Burnet, F.M., and Bull, D.R., Re-examination of the influenza virus strain "Melbourne egg," Australian J. Exper. Biol. and Med., 22, 173-178, 1944.

27. Francis, T., Apparent serological variation within a strain of influenza virus, Proc. Soc. Exper. Biol. and Med. 65, 143-147, 1947.

28. Hirst, G.K., Strain specific elements in influenza antigens, J. Exper. Med., 96, 589-603, 1952.

29. Jensen, K.E., and Francis, T., The antigenic composition of influenza virus measured by antibody absorption, J. Exper. Med., 98, 619-639, 1953.

30. Choppin, P.W., and Tamm, I.J., Two kinds of particles with contrasting properties in influenza A_2 virus strains from the 1957 pandemic, Virology, 8, 539-542, 1959.

31. Choppin, P.W., and Tamm, I.J., Studies on two kinds of virus particles which comprise influenza A_2 virus strains: I Characterization of stable homogeneous substrains in reactions with specific antibody, mucoprotein inhibitors, and erythrocytes, J. Exper. Med., 112, 895-920, 1960.

32. Lief, F.S., Toxicity, pathogenicity and antigenicity of inhibitor-sensitive and non-sensitive substrains of influenza A_2 isolates, J. Immunol., 92, 286-298, 1964.

33. Wenner, H.A., Kamitsuka, P., and Lanahan, M., A comparative study of type II polio viruses; II Antigenic differences relating to 18 type II strains, J. Immunol., 77, 220-231, 1956.

34. Meyer, K.F., Haring, C.M., and Howitt, B., Newer knowledge of the neurotropic virus infections of the horse, J. Am. Veterinary Med. Assn., 79, 376-389, 1931.

35. Meyer, K.F., Haring, C.M., and Howitt, B., The etiology of epizootic encephalomyelitis of horses in the San Joaquin Valley, 1930, Science, 74, 227-228, 1931.

36. Howitt, B., Recovery of the virus of equine encephalomyelitis from the brain of a child, Science, 88, 455-456, 1938.

37. Meiklejohn, G., and Hammon, W. McD., Epidemic of encephalitis, predominately St. Louis type in Pinal County, Arizona, J. Am. Med. Assn., 118, 961-964, 1942.

38. Sulkin, S.E., Recovery of equine encephalomyelitis virus (western type) from chicken mites, Science, 101, 381-383, 1945.

39. Hammon, W. McD., Reeves, W.C., and Galendo, P., Epidemiological studies of encephalitis in the San Joaquin Valley of California, 1943, with the isolation of virus from mosquitoes, Am. J. Hyg., 42, 299-306, 1945.

40. Hammon, W. McD, Reeves, W.C., Benner, S.R., and Brookman, B., Human encephalitis in the Yakima Valley, Washington, 1942, with forty-nine virus isolations (western equine and St. Louis types) from mosquitoes, J. Am. Med. Assn., 128, 1133-1139, 1945.

41. Eklund, C.M., Human encephalitis of the western equine type in Minnesota in 1941: clinical and epidemiological study of serologically positive cases, Am. J. Hyg., 43, 171-193, 1946.

42. Reeves, W.C., Mack, W.N., and Hammon, W. McD., Epidemiological studies on western equine encephalitis and St. Louis encephalitis in Oklahoma, 1944, J. Inf. Dis., 81, 191-196, 1947.

43. Lennette, E.H., Magoffin, R.L., Longshore, W.A., Jr., and Hollister, A.C., Jr., An etiological study of seasonal aseptic meningitis and encephalitis in the Central Valley of California, Am. J. Trop. Med. and Hyg., 10, 885-896, 1961.

44. LaVeck, G.D., Winn, J.F., and Welch, S.F., Inapparent infection with western equine encephalitis virus: epidemiological observations, Am. J. Pub. Health, 45, 1409-1416, 1955.

45. Holden, P., Recovery of western equine encephalitis from naturally infected English sparrows of New Jersey, 1953. Proc. Soc. Exper. Biol. and Med. 88, 490-492, 1955.

46. Paull, A.M., and Young, R., Western equine encephalomyelitis in Rhode Island, Rhode Island Med. J., 43, 633-635,

47. Kelser, R.A., Mosquitoes as vectors of the virus of equine encephalomyelitis, Am. J. Veterinary Med. Assn., 82, 767-771, 1933.

48. Grundmann, A.W., Kitselman, C.M., Roderick, L.M., and Smith, R.C., Studies on the transmission of the western strain virus of equine encephalitis by the American dog tick Dermacentor variablis Say and by Triatoma sanguisuga (Le Conte), J. Inf. Dis., 72, 163-171, 1943.

49. Hammon, W. McD., Reeves, W.C., Brookman, B., Izumi, E.M., and Gjullin, C.M., Isolation of the viruses of western equine encephalitis and St. Louis encephalitis from Culex tarsalis mosquitoes, Science, 94, 328-330, 1941.

50. Hammon, W. McD., Reeves, W.C., Brookman, B., and Izumi, E.M., Mosquitoes and encephalitis in the Yakima Valley, Washington: I Arthropods tested and recovery of western equine and St. Louis viruses from Culex tarsalis Coquillett, J. Inf. Dis., 70, 263-266, 1942.

51. Burroughs, A.L., and Burroughs, R.N., A study of the ecology of western equine encephalitis virus in the upper Mississippi River Valley, Am. J. Hyg., 60, 27-36, 1954.

52. Reeves, W.C., Bellamy, R.E., and Scrivani, R.P., Relationships of mosquito vectors to winter survival of encephalitis viruses: I. Under natural conditions, Am. J. Hyg., 67, 78-89, 1958.

53. Rush, W.A., Kennedy, R.C., and Eklund, C.M., Evidence against maintenance of western equine encephalitis virus by Culex tarsalis during spring in northwestern United States, Am. J. Hyg., 77, 258-264, 1963.

54. Blackmore, J.S., and Winn, J.F., A winter isolation of western equine encephalitis virus from hibernating Culex tarsalis Coquillett, Proc. Soc. Exper. Biol. and Med., 91,146-148, 1956.

55. Reeves, W.C., Hammon, W. McD., Lazarius, A.S., Brookman, B., McClure, H.E., and Doetschman, W.H., The changing picture of encephalitis in the Yakima Valley, Washington, J. Inf. Dis., 90, 291-301, 1952.

56. Hammon, W. McD., Lundy, H.W., Gray, J.A., Evans, F.C., Bang, F., and Izumi, E.M., A large-scale serum neutralization survey of certain vertebrates as part of an epidemiological study of encephalitis of the western equine and St. Louis types, J. Immunol., 44, 75-86, 1942.

57. Burton, A.N., Connell, R., Rempel, J.G., and Gollop, J.B., Studies on western equine encephalitis associated with wild ducks in Saskatchewan, Canad. J. Microbiol., 7, 295-302, 1961.

58. Kaplan, W., Winn, J.F., and Palmer, D.F., Susceptibility of the pigeon (Columbia livia) to infection with western equine encephalitis virus, J. Immunol., 75, 225-226, 1955.

59. Reeves, W. C., Tempolis, C.A., Bellamy, R.E., and Lofy, M.F., Observations of the feeding habits of Culex tarsalis in Kern County, California, using precipitating antisera produced in birds, Am. J. Trop. Med. and Hyg., 12, 929-934, 1963.

60. Kissling, R.E., Chamberlain, R.W., Sudia, W.D., and Stamm, D.D., Western equine encephalitis in wild birds, Am. J. Hyg., 66, 48-55, 1957.

61. Hammon, W. McD., and Reeves, W.C., Recent advances in the epidemiology of the arthropod-borne virus encephalitides including certain exotic types, Am. J. Pub. Health, 35, 994-1004, 1945.

62. Chamberlain, R.W., Sikes, R.K., Nelson, D.B., and Sudia, W.D., Studies on the North American arthropod-borne encephalitides: VI Quantitative determination of virus-vector relationships, Am. J. Hyg., 60, 278-285, 1954.

63. Reeves, W.C., Hutson, G.A., Bellamy, R.E., and Scrivani, R.P., Chronic latent infections of birds with western equine encephalitis virus, Proc. Soc. Exper. Biol. and Med. 97, 733-736, 1958.

64. Winn, J.F., and Palmer, D.F., Recovery of western equine encephalitis virus from crop washings of experimentally infected pigeons, Am. J. Veterinary Res., 22, 139-141, 1961.

65. Kissling, R.E., Stamm, D.D., Chamberlain, R.W., and Sudia, W.D., Birds as winter hosts for eastern and western equine encephalomyelitis viruses, Am. J. Hyg., 66, 42-47, 1957.

66. Syverton, J.T., and Berry, G.P., An arthropod vector for equine encephalomyelitis, western strain, Science, 84, 186-187, 1936.

67. Syverton, J.T., and Berry, G.P., Hereditary transmission of the western type of equine encephalomyelitis virus in the wood tick, Dermacentor andersoni Stiles, J. Exper. Med., 73, 507-529, 1941.

68. Thomas, L.A., and Eklund, C.M., Overwintering of western equine encephalitis virus in experimentally infected garter snakes and transmission to mosquitoes, Proc. Soc. Exper. Biol. and Med., 105, 53-55, 1960.

69. Karabatos, N., Bourke, A.T.C., and Henderson, J.R. Antigenic variation among strains of western equine encephalitis virus, Am. J. Trop. Med. and Hyg., 12, 408-412, 1963.

70. Niederman, J., Paul, J., Pearson, J., and Florey, C., Serum survey in recruits in the Armed Forces, To be published.

71. Casals, J., Palacios, R., Diagnosis of epidemic encephalitis by complement-fixation test, Science, 94, 330, 1941.

72. Clarke, D.H., and Casals, J., Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses, Am. J. Trop. Med. and Hyg., 7, 561-573 1958.

73. Henderson, J.R., and Taylor, R.M., Propagation of certain arthropod-borne viruses in avian and primate cell cultures, J. Immunol., 84, 590-598, 1960.

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